TECHNICAL NOTES

SIMPLE ON-COLUMN TRAPPING PROCEDURE FOR GAS CHROMATOGRAPHIC ANALYSIS OF FLAVOR VOLATILES[1]

Primary analyses of flavor volatiles commonly involve distillation of large samples, concentration of distillates, tentative indentification of components by gas liquid chromatography (GLC) and ultimate positive identification by derivative characterization, and spectrometric techniques such as infrared, UV, or mass spectrometry. Such involved procedures obviously are not suited for rapid routine evaluation of product aroma, nor should they be necessary once the identity of the principal volatile components has been established. The volatile constituents in a confined space over a food product should be present in concentration proportional to their respective partial pressures; thus, with suitable sampling and sufficiently sensitive analyses of such vapors, it should be possible to detect both qualitative and quantitative changes in aroma composition.

In our hands, syringe sampling of head-space vapors for GLC analyses (1) has lacked precision, and the inherent limitation of sample size has prohibited its use in analyses of products possessing rather weak aromas. The technique of on-column trapping of entrained volatiles as described by Hornstein and Crowe (3) appears to have overcome the latter limitation. Bingham's (2) experience with this technique in the analysis of vapors over reconstituted concentrated milks has prompted us to make certain modifications in adapting it to current studies involving the direct analyses of the volatiles from butter, margarine, cheese fat, concentrated milks, and milk cultures of microorganisms.

Screw-capped vials (Kimble no. 60957, size no. 1) charged with sufficient granular anhydrous Na_2SO_4 to saturate the aqueous portion of the samples to be analyzed are placed in a forced-draft oven at 105 C overnight, then stored sealed with aluminum foil under the original caps. Samples (5-10 ml) are pipetted or weighed into the vials and, if necessary, a few milligrams of 1-tetradecanol may be added to control foaming. The original caps are replaced with ones in which two 5/32-in. holes 7/16-in. apart have been drilled and the original liners replaced with ones of 1/8-in.-thick silicone rubber. The latter may be cut from sheet stock with a no. 15 cork borer.

The entrainment assembly (Figure 1), rigidly supported alongside the chromatographic oven as pictured in Figure 2, consists of two 22-

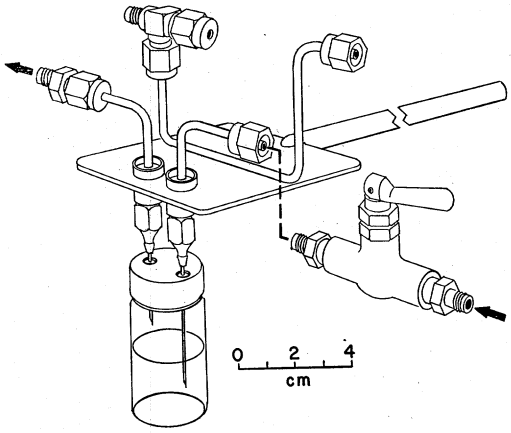

FIG. 1. Details of entrainment apparatus.

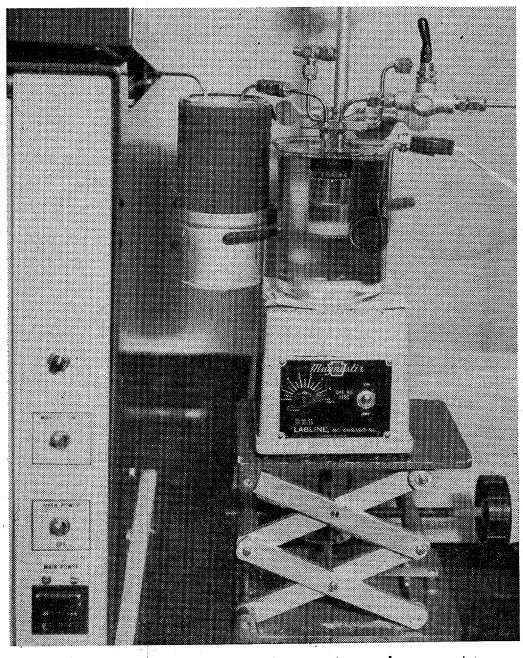

FIG. 2. Assembled on-column trapping system.

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gauge syringe needles (2 in. and ½-in. Hamilton N-722, point style no. 1) held rigidly parallel 7/16-in. apart in Luer-Lok fittings (salvaged from discarded syringe barrels) through which the entraining gas is conducted into and from the sample vial. In construction, the syringe ends of these fittings were drilled to accept short pieces of ⅛-in. od stainless steel tubing, these silver-soldered in place and the fittings then soldered in appropriate holes in the support plate. The bends in the tubing were made and ⅛-in. Swagelok fittings installed as indicated. Gaskets cut from ⅛-in. silicone rubber with no. 3 and no. 1 cork borers were forced into the Luer-Lok fittings to provide a leak-proof seat against which the needles could be screwed in place.

The vial containing the sample is tempered at the desired entrainment temperature with intermittant vigorous agitation. A clean, empty vial is then clamped in place so that it shields both needles. Nitrogen, from a tank regulator set at a pressure predetermined to give a flow rate of 10 cc per minute through a 6-ft section of ¼-in. tubing packed with molecular sieve 5A, the assembled entrainment system, and the GLC column, is turned on by opening the valve shown in Figure 1. With the GLC instrument conditions adjusted to those under which the analysis of the trapped volatiles is to be made, the instrument carrier gas is shut off with a toggle valve installed in the line just ahead of the injection port. The column, in which a U-shaped section (3 in. deep) has been formed just beyond the column fitting, is disconnected from the injection port, stretched over the side of the oven, and connected to the entrainment apparatus. The oven cover is closed as far as the column will permit and blocked open at that point. A Dewar flask containing a slurry of dry ice in 2-methoxyethanol is raised so that the trap portion of the column is immersed and is clamped in position as shown in Figure 2. Since the short effluent needle is bathed in nitrogen at this point, volatiles from the room atmosphere cannot be aspirated and condensed in the column trap. When the trap has been cooled, the shielding vial is removed and the septum on the tempered sample vial punctured with both needles, inserted to their full length. A small waterbath controlled at the desired temperature is raised so that the sample vial and needle assembly are immersed to the horizontal slits on the sides of the Luer-Lok fittings. Nitrogen is allowed to bubble up through the sample for the desired time, thus carrying the entrained volatiles into the column trap, where they are condensed on the column packing. Condensation of volatiles in the tubing and fittings between the effluent needle and the column can be prevented by heating this section with hot air from a heat gun supported over the apparatus.

With the waterbath lowered, the sample vial is pulled from the needles at the end of the entrainment period. In quick succession the column is disconnected from the entrainment assembly, connected to the injection port, and the oven closed. After allowing a few moments for the oven temperature to equilibrate, the carrier gas is turned on, and the chromatogram developed and recorded in the usual manner.

With the toggle valve on the nitrogen line connected to the U-shaped tube on the support plate (Figure 1) and the column trap connected to the injection tee, known compounds or mixtures thereof may be injected into and condensed on the column for determination of retention times.

We have found it convenient to use, alternately, several of the devices shown in Figure 1, rinsing each thoroughly with acetone and either after use and storing them in a hot-air oven. This precludes transfer of traces of material from previous samples to the column trap.

In our present applications we have used an F & M Model 810 GLC instrument equipped with dual-flame ionization detectors, which permits alternate use of two identical or different columns. Obviously, the technique as described requires an instrument with an oven from which the trap end of the column may be readily removed for the entrainment step and one which possesses a low mass and a heating system capable of rapid equilibration. With the present oven set to operate at temperatures from 40 to 90 C, the temperature does not drop more than 0.5 C during the time the column is in the entrainment position and returns to the operating temperature within a minute after the column trap is returned to the oven and the cover closed.

Thus far, 12 ft by ⅛-in. stainless steel columns packed with 60-80 mesh acid and alkali-washed Celite coated with 20% 1,2,3-*tris* (2-cyanoethoxy) propane have been found to be most useful with this technique in our applications. The flow rate of nitrogen carrier gas through such columns has been adjusted to 24-30 cc per minute at the desired column-operating temperature. Undoubtedly, other columns and operating conditions could be employed.

Entrainment of volatiles from samples with from 50 to 100 cc of nitrogen at sample temperatures of 60 to 90 C has provided sufficient material so that even minor components are detected at a sensitivity setting at which 1 mv full-scale recorder deflection requires approximately 4×10^{-11} amp (range 10 and $\times 1$ attenuation on the F & M 810 instrument). A further fivefold increase in sensitivity is possible, but a small, flat peak which appears in both blank and sample chromatograms is elevated at this sensitivity. Apparently, this is

TABLE 1

Precision of chromatographic analyses of known compounds entrained from aqueous and nonaqueous samples

Mixture	Distilled water saturated with Na_2SO_4 [a]			Stripped milk fat [b]		
	Conc	Mean peak height [c]	Retention distance [d]	Conc	Mean peak height [c]	Retention distance [d]
	(ppm)	*(mm)*	*(mm)*	*(ppm)*	*(mm)*	*(mm)*
Acetaldehyde	0.05	114.57 ± 3.48	29– 31	0.10	118.00 ± 3.46	30– 31
Ethyl acetate	0.05	318.86 ± 21.63	68– 70	0.50	192.71 ± 35.90	68– 71
Butanone	0.10	358.29 ± 10.62	101–102	0.50	164.14 ± 8.59	100–103
Diacetyl	0.10	54.40 ± 4.04	160–162	1.00	94.00 ± 4.45	158–163

[a] Eight-milliliter sample, entrained with N_2 at 10 cc per minute for 5 min at 60 C.

[b] Eight grams fat, entrained with N_2 10 cc per minute for 10 min at 60 C.

[c] Means and standard derivations calculated on seven trials.

[d] Column temp, 70 C; N_2 flow rate, 24 cc per minute.

due to a momentary accumulation of bleed in the column during the time the carrier gas flow is interrupted. This aberration interferes in only a relatively small portion of the chromatogram and does not preclude further increases in sensitivity before or after emergence of this peak. Under these conditions, we have experienced no difficulty in detecting differences in a variety of compounds in dairy products which range in volatility from that of methyl sulfide to those of 2-heptanone, ethyl octanoate, and 3-methylbutanol.

By maintaining a slow flow rate and keeping the total volume of entrainment gas low, we have not found it necessary to increase the length of the column trap or to employ liquid nitrogen to insure complete condensation of volatiles, nor have we found it necessary to remove water from entrained volatiles (1).

The reproducibility of replicate chromatograms of mixtures of acetaldehyde, ethyl acetate, butanone, and diacetyl entrained from very dilute solutions in distilled water saturated with Na_2SO_4 and stripped milk fat is shown in Table 1. When entrained from the aqueous sample, the recoveries of acetaldehyde and butanone were reproducible to approximately ± 3%, whereas those for ethyl acetate and diacetyl were about ± 7%. As expected, the entrainment of these compounds from milk fat was somewhat more difficult; thus, the concentration of each component was increased and the entrainment time doubled to give readily measurable peaks at the same sensitivity (range 10, ×1 attenuation). Under these conditions reproducibility of the recoveries of acetaldehyde, butanone, and diacetyl were ± 5% or less; whereas, the recovery of ethyl acetate fluctuated markedly (19%). The reason for the variability in recovery of ethyl acetate is not readily apparent, but it is probably related to its infinite solubility in the fat and incomplete equilibration of this component in the sample head space prior to entrainment. It is likely that extending the equilibration time from that used in the present trials (8-10 min) would result in greater precision of recovery (4).

We have employed the technique described above in conjunction with rapid-scan mass spectrometry of split GLC column effluent. The Atlas CH-4 equipped with the EC-1 inlet system was used. This combination of technique has proved to be highly successful and provides a means for immediate positive identification of volatile constituents.

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